

Control of Directionality in Bacteriophage mv4 Site-Specific Recombination: Functional Analysis of the Xis Factor[∇]

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The integrase of the temperate bacteriophage mv4 catalyzes site-specific recombination between the phage *attP* site and the host *attB* site during *Lactobacillus delbrueckii* lysogenization. The mv4 prophage is excised during the induction of lytic growth. Excisive site-specific recombination between the *attR* and *attL* sites is also catalyzed by the phage-encoded recombinase, but the directionality of the recombination is determined by a second phage-encoded protein, the recombination directionality factor (RDF). We have identified and functionally characterized the RDF involved in site-specific excision of the prophage genome. The mv4 RDF, ^{mv4}Xis, is encoded by the second gene of the early lytic operon. It is a basic protein of 56 amino acids. Electrophoretic mobility shift assays demonstrated that ^{mv4}Xis binds specifically to the *attP* and *attR* sites via two DNA-binding sites, introducing a bend into the DNA. *In vitro* experiments and *in vivo* recombination assays with plasmids in *Escherichia coli* and *Lactobacillus plantarum* demonstrated that ^{mv4}Xis is absolutely required for inter- or intramolecular recombination between the *attR* and *attL* sites. In contrast to the well-known phage site-specific recombination systems, the integrative recombination between the *attP* and *attB* sites seems not to be inhibited by the presence of ^{mv4}Xis.

During the establishment of lysogeny, many temperate bacteriophages integrate their genomes into the bacterial host chromosome in a site-specific manner: recombination between the attachment sites *attB* and *attP*, located on the bacterial and phage genomes, respectively, is catalyzed by a phage-encoded recombinase (integrase) (14, 42) and leads to formation of the hybrid attachment sites *attL* and *attR* at the junctions between the phage and bacterial genomes. Prophage excision, which occurs during the induction of lytic growth, involves site-specific recombination between the attachment junctions *attL* and *attR* and requires at least one phage-encoded recombination directionality factor (RDF) or excise, in addition to integrase (32, 43).

In the model *Escherichia coli* phage λ and its close relatives, the recombination directionality is controlled directly by the RDF Xis. The gene *xis* is located upstream from the *int* gene encoding the integrase. Xis is required for the excision reaction but inhibits integrative recombination (2, 41). Xis determines the directionality of recombination by influencing the formation of specific protein-DNA architectures (30). To exert its effect, Xis binds cooperatively with Int and FIS to specific sites, thereby inducing and stabilizing a DNA bend that alters the intasome structure formed during recombination (1, 15, 44, 53).

In bacteriophages of the P2 family, site-specific recombination-related genes are organized differently, even though the directionality of recombination seems to be controlled as in phage λ . The P2 genetic switch region contains two overlapping face-to-face promoters, *p_c* and *p_e* (50). The leftward pro-

motor, *p_c*, produces the lysogenic transcript, including the C repressor and P2 integrase, whereas the rightward promoter, *p_e*, produces the lytic transcript. The first protein encoded by the lytic operon, Cox, is a λ Cro-like repressor of the promoter *p_c* controlling lysogeny (50). However, Cox is a multifunctional protein that also acts as a recombination directionality factor (57).

Site-specific recombination has been well studied for bacteriophages of Gram-positive bacteria, essentially for bacteriophages of *Actinomycetales*, such as *Mycobacterium* phage L5 (36, 46–48) and *Streptomyces* phage ϕ C31 (52, 55), but also for bacteriophages of lactic acid bacteria (3, 10, 11, 22, 49). However, only few RDFs have been characterized to date, for example, those of mycobacteriophage L5 (34, 36) and of lactococcal phage TP901-1 (9).

The site-specific integration system of bacteriophage mv4 has been characterized previously (6, 22). The mv4 integrase belongs to the tyrosine recombinase family and has no absolute requirement for accessory factors, unlike most of the λ Int family recombinases (4). The mv4 integrase also functions in a wide range of Gram-positive bacteria and in *Escherichia coli* (6). Furthermore, a site-specific integrative vector derived from mv4 is stably maintained in the chromosome of several lactobacilli in the absence of selection, suggesting that ^{mv4}Int cannot catalyze excisive recombination on its own (6). The minimal 234-bp *attP* site of mv4 contains five putative integrase arm-binding sites and has a 17-bp core sequence in common with the *attB* site (4). During recombination, strand exchange occurs inside the core sequence. The *attB* site is located at the 3' end of a tRNA^{ser} gene (22). The minimal size of the functional *attB* site is 16 bp, and no symmetric inverted repeats have been found in the core sequence (5).

In this study, we identified the RDF gene ^{mv4}*xis* as the second gene of the mv4 early lytic operon. This location is

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TABLE 1. Bacteriophage, bacterial strains, and plasmids used in this study

| Strain/phage/plasmid | Relevant characteristic(s) | Reference/source |
|----------------------|---|-------------------|
| Strains | | |
| <i>E. coli</i> | | |
| DH5 α | F ⁻ ϕ 80dlacZ Δ M15 Δ (lacZYA-argF)U169 endA1 λ^- hsdR17(r _K ⁻ m _K ⁺) deoR thi-1 supE44 recA1 gyrA96 relA1 | Life Technologies |
| TG2 | supE hsd Δ 5 thi Δ (lac-proAB) Δ (srl-recA)306::Tn10 (Tet ^r) F' [traD36 proAB ⁺ lacI ^a lacZ Δ M15] | 51 |
| BL21(DE3) | F ⁻ omp T hsdS _B (r _B ⁻ m _B ⁻) gal dcm (DE3) | Novagen |
| <i>L. plantarum</i> | | |
| LP80 | <i>Lactobacillus plantarum</i> DSM 4229 | 29 |
| LP80-3 | LP80::pMC1 integrant | 22 |
| Bacteriophage mv4 | Temperate phage isolated from the <i>L. delbrueckii</i> LT4 strain | 16 |
| Plasmids | | |
| pRC1 | ermAM ⁺ (Em ^r), 3.5-kb cloning vector, ColE1 replicon | 33 |
| pMC1 | Em ^r , 5.15 kb, pRC1 with mv4 attP-int region | 22 |
| pRC10 | Em ^r , 3.85 kb, pRC1 with mv4 attP region | 4 |
| pRCattR | Em ^r , 5.2 kb, pRC1 with attR region cloned at the SalI and XbaI sites | This work |
| pRCattRDR2 | Em ^r , 5.2 kb, pRC1 with a deletion derivative of the attR region cloned at the BamHI and KpnI sites | This work |
| pRCattL | Em ^r , 4 kb, pRC1 with attL region cloned at the SalI and XbaI sites | This work |
| pMPM-A3 | bla ⁺ (Amp ^r), 3.4-kb cloning vector, p15A replicon | 40 |
| pA3int | Amp ^r , 4.75 kb, pMPM-A3 with mv4 int gene | 4 |
| pNZ8037 | cat ⁺ (Cm ^r), 3-kb expression vector, pSH71 replicon | 20 |
| pNZ57 | Cm ^r , 3.65 kb, pNZ8037 with mv4 ORF-57 cloned at the SmaI site | This work |
| pNZ109 | Cm ^r , 3.6 kb, pNZ8037 with mv4 ORF-109 cloned at the BamHI and SmaI sites | This work |
| pNZ179 | Cm ^r , 3.6 kb, pNZ8037 with mv4 ORF-179 cloned at the SmaI and PstI sites | This work |
| pNZxis | Cm ^r , 3.2 kb, pNZ8037 with mv4 ORF-56 cloned at the SmaI site | This work |
| pNZxish | Cm ^r , 3.2 kb, pNZ8037 with mv4 ORF-56 His tagged at the C terminus cloned at the BamHI and EcoRI sites | This work |
| pNZ9530 | Em ^r , 7 kb, pIL252 carrying nisRK (pAM β 1 replicon) | 31 |
| pAM239 | aad9 (Spc ^r), 4.3-kb cloning vector, pSC101 replicon | D. Gil |
| pAMattB | Spc ^r , 4.6 kb, pAM239 with the attB region | 4 |
| pAMattR | Spc ^r , 4.6 kb, pAM239 with the attR region cloned at the BamHI and SmaI sites | This work |
| pET15b | bla ⁺ (Amp ^r), 5.7-kb expression vector, pBR322 replicon | Novagen |
| pETxish | Amp ^r , 5.9 kb, pET15b with mv4 ORF-56 His tagged at the C terminus cloned at the NcoI and XhoI sites | This work |
| pCointRL | Em ^r , Spc ^r , 8.45 kb, cointegrate between pAMattB and pRC10 bearing the attR and attL sites | This work |

unusual, being reported here for the first time in a temperate bacteriophage harboring a tyrosine recombinase. mv4Xis is a 56-residue basic peptide that acts by binding to two sites within attR or attP DNA, thereby bending the DNA. The presence of mv4Xis is absolutely required, together with mv4Int, for excisive recombination between the attL and attR attachment sites. However, in contrast to results for the well-studied systems (λ , L5, HP1, and P2 phages), preliminary results suggest that mv4Xis does not inhibit integrative recombination between the attP and attB sites.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and growth conditions. The bacterial strains, bacteriophage, and plasmids used in this study are listed in Table 1. *Lactobacillus plantarum* strains were grown at 37°C in MRS medium (19) or on MRS medium solidified with 1.5% (wt/vol) agar. Erythromycin and chloramphenicol were used at concentrations of 5 μ g/ml and 10 μ g/ml, respectively. *E. coli* was grown at 37°C in Luria-Bertani broth (Difco Laboratories, Detroit, MI) or on LB broth solidified with 1.5% (wt/vol) agar. Antibiotics were used at the following concentrations: 100 μ g/ml for ampicillin, 150 μ g/ml for erythromycin, 10 μ g/ml for chloramphenicol, and 65 μ g/ml for spectinomycin.

DNA techniques. DNA techniques were performed essentially as described by Sambrook et al. (51). Restriction enzymes, the Klenow fragment of DNA polymerase I, Taq polymerase, T4 polynucleotide kinase, and T4 DNA ligase were

purchased from either Roche Molecular Biochemicals (Mannheim, Germany) or New England Biolabs (Beverly, MA) and used as recommended by the suppliers.

E. coli and *L. plantarum* were electrotransformed using a gene pulser device (Bio-Rad, Richmond, CA) according to the manufacturer's recommendations and according to the method of Auvray et al. (6), respectively.

E. coli plasmid DNA was isolated using a Qiaprep spin kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Total DNA was obtained from *L. plantarum* and *E. coli* as previously described (22).

Plasmid construction. The plasmids used in this work are listed in Table 1 and the primers (Eurogentec, Seraing, Belgium) in Table 2. The 1,757-bp SalI-XbaI fragment (containing the attR region) of pBSattR (22) was ligated to the 3,438-bp SalI-XbaI fragment of pRC1 to give pRCattR. The 590-bp SalI-XbaI fragment (containing the attL region) of pBSattL (22) was ligated to the 3,438-bp SalI-XbaI fragment of pRC1 to give pRCattL. The 278-bp BamHI PCR fragment, amplified from pRCattR using the DR9 and attb primers, was ligated to the 4,330-bp BamHI-SmaI fragment of pAM239 to give pAMattR. The 636-bp PCR fragment, amplified from mv4 DNA using the F3 and F4 primers, was ligated to the 3,016-bp SmaI fragment of pNZ8037 to give pNZ57. The 588-bp BamHI-XmnI PCR fragment, amplified from mv4 DNA using the C1 and BX1 primers, was ligated to the 3,010-bp BamHI-XmnI fragment of pNZ8037 to give pNZ109. The 216-bp BamHI-EcoRI PCR fragment, amplified from pNZxis (pNZxis was constructed by inserting a 224-bp SmaI-XmnI fragment from mv4 DNA containing the xis gene into the SmaI site of pNZ8037) using the Xis1 and XisHis primers, was ligated to the 3,001-bp BamHI-EcoRI fragment of pNZ8037 to give pNZxish. The 243-bp NcoI-XhoI PCR fragment, amplified from pNZxis using the Xis1 and XisHis primers, was ligated to the 5,643-bp NcoI-XhoI fragment of pET15b to give pETxish. pCointRL is the cointegrate resulting from site-specific

TABLE 2. List of primers used in this study

| Primer | Sequence (5' → 3') ^a | Restriction site(s) |
|--------|--|----------------------|
| DR2 | GGGGTACCAGAAACGCTTTTATAGC | KpnI |
| DR3 | ATGCCATCTATTAAGTAGCT | |
| DR4 | AACTGCAGTGAACGCATGGAAACA | PstI |
| DR9 | CGGGATCCAGTTCTAAATCAACTAG ATTTTAACT | BamHI |
| BX1 | GCATCTGGATCTTAT | |
| C1 | GCATCTGGATCTTATAATTA | |
| F3 | TCACCCGGGCAAGATCATCG | SmaI |
| F4 | TTTGACTGCAGGATTACGGC | PstI |
| attb | CATTGTATTTAGATGTCCTT | |
| L1 | CACCATCTTAAAAATAACTT | |
| Xis1 | TCACCATGGTACTGCACTGGATCCC | NcoI, BamHI |
| XisHis | ACTCGAGTCTAGAATTCCTAGTGAT GGTGATGGTGATGTTCTCTCT TAAA | XhoI, XbaI, EcoRI |
| X1mut | AAACGCAGCA <u>ACCCG</u> ACCAGA | |
| X2mut | AACCCTCGCA <u>ACCCG</u> TGGTTG | |
| X2A | CCAGAAACATGGTTGAAAGAACC | |
| X2B | ATGTTTCTGGAGGGTCTGG | |
| Uni | GTAAAACGACGGCCAGT | |
| Rev | GGAAACAGCTATGACCATG | |

^a The restriction sites contained in the primers are underlined. The mutations are in bold and underlined (for X1mut and X2mut). The Xis binding site sequences are in bold (for X2A and X2B).

recombination between pRC10 (*attP*) and pAMattB in the *E. coli* TG2 strain in the presence of pA3int. pCointRL was purified from agarose gel and introduced by transformation into the TG2 strain for amplification.

Construction of the *attR* site deletion derivative. The mv4 *attR* DR2 DNA segment was amplified by PCR, using DR2 and Uni oligonucleotides and pRCattR as the template. The amplified fragment was cut with BamHI and KpnI and purified by polyacrylamide gel electrophoresis, and the 1,655-bp BamHI-KpnI fragment was ligated to the 3,461-bp BamHI-KpnI fragment of pRC1 to give pRCattRDR2.

Protein production and preparation. Cell extracts containing soluble mv4Xis were prepared from *L. plantarum* LP80 cells carrying pNZxish and pNZ9530 (*nisRK*). Control crude extract without mv4Xis was prepared from *L. plantarum* LP80 cells carrying pNZ8037 (empty expression vector) and pNZ9530. Overnight cultures were diluted 1/50 in MRS and grown at 37°C. When the cultures reached an optical density at 600 nm (OD₆₀₀) of 0.2, nisin was added to a final concentration of 25 ng/ml. The cells were collected by centrifugation 5 h after nisin addition; washed three times with 50 mM Tris-HCl, pH 7.5, 10% glycerol; and incubated in a 1/20 volume of lysis buffer (50 mM Tris-HCl, pH 7.5, 10% glycerol, 0.1 mM phenylmethylsulfonyl fluoride [PMSF], 1 mg/ml lysozyme) overnight at 4°C. The cells were sonicated 10 times, for 30 s each, on ice and stored for 1 h at 4°C with 1% Triton X-100. Cell debris was removed by centrifugation at 3,000 × *g* for 1 h at 4°C. *E. coli* preparations containing soluble mv4Int were obtained as previously described (4). The protein concentrations of extracts were determined by the Bradford assay (8).

Gel electrophoretic mobility shift assay (GEMSA). The primers were end labeled using polynucleotide kinase and [γ -³²P]ATP (Amersham) (51) before amplification of the 242-bp DR9-DR3 (*attP*), 281-bp DR9-attb (*attR*), 244-bp L1-DR3 (*attL*), 283-bp L1-attb (*attB*), 207-bp DR4-DR3 (*attPD4*), 187-bp DR2-DR3 (*attPD2*), 197-bp X1mut-DR3 (*attPX1mut*), and 137-bp X2mut-DR3 (*attPX2mut*) DNA fragments with pRC10, pRCattR, pRCattL, or pAMattB as the template. The 187-bp DR2-DR3 fragment was digested with BstBI or BstXI, and the resulting 149-bp (*attPBB*) or 123-bp (*attPBX*) fragment, respectively, was purified from the acrylamide gel. DNA binding reactions were performed with a reaction volume of 20 μ l containing 30 mM Tris-HCl, pH 7.5, 5 mM NaCl, 90 mM KCl, 5 mM EDTA, 1% glycerol, 2 μ g poly(dI-dC), 10⁵ cpm of probe, and 10 μ g of protein for 10 min at room temperature before electrophoresis. Protein-DNA complexes were separated by electrophoresis with 5% nondenaturing polyacrylamide gels. The gels were run in TBE buffer (0.1 M Tris-HCl, pH 8, 0.1 M borate, 0.7 mM EDTA). The radioactive compounds were detected with a Fuji BAS1000 bioimaging analyzer system (Fuji Photo Film Co., Tokyo, Japan) and analyzed with Tina version 2.07c software (Raytest Isotopenmeßgeräte GmbH,

Straubenhardt, Germany) and/or by autoradiography after the gels were placed against BioMax MR film (Kodak) at room temperature.

Bending. The 552-bp Uni-Rev, 182-bp X2A-Rev, and 216-bp Uni-X2B DNA fragments were radioactively labeled during amplification, using 20 μ Ci [α -³²P]dATP per reaction mixture and pRCattRDR2 as the template. The 181-bp AccI-BstBI, 182-bp KpnI-MslII, 180-bp SpeI-XhoI, 181-bp AccI-XbaI, and 185-bp HindIII-SacI fragments were purified from acrylamide gels after digestion of the 552-bp Uni-Rev fragment with the corresponding restriction enzymes. The 180-bp SfcI-X2B fragment was purified from an acrylamide gel after digestion of the 216-bp Uni-X2B fragment with SfcI. DNA-protein complex analysis by electrophoresis was carried out as described above for GEMSA.

Preparation of radioactive substrates. The 283-bp L1-attb (*attB*), 281-bp DR9-attb (*attR*), 244-bp L1-DR3 (*attL*), and 242-bp DR9-DR3 (*attP*) DNA fragments were radioactively labeled during amplification, using 20 μ Ci [α -³²P]dATP per reaction mixture.

In vitro excisive recombination assays. *attL* × *attR* intermolecular recombination reaction mixtures (20 μ l), containing 200 ng supercoiled plasmid carrying the *att* site to be tested and a linear radiolabeled *att* fragment (10⁵ cpm) in 30 mM Tris-HCl, pH 7.5, 5 mM NaCl, 90 mM KCl, 5 mM EDTA, 1% glycerol, and 4 mM spermidine, were incubated for 90 min at 42°C in the presence or absence of Int cell extracts (3 μ g protein equivalent) and with Xis or control cell extracts (3 μ g or 6 μ g protein equivalent, respectively). The reactions were stopped by adding 0.1% sodium dodecyl sulfate (SDS) and dye. For time course experiments, aliquots were removed at various time points and the reaction was stopped. Samples were analyzed as previously described (4).

In vivo site-specific recombination test with *E. coli*. *E. coli* recA TG2 cells carrying pA3int and (i) pRC10 and pAMattB, (ii) pCointRL, or (iii) pRCattL and pAMattR were transformed with pNZ8037 or pNZxish. Cells were cultured in the presence of appropriate antibiotics for about 25 generations, and the plasmids were then extracted. After restriction digestion, the plasmids were analyzed by agarose gel electrophoresis with ethidium bromide staining.

Nucleotide sequence accession numbers. The sequence data have been submitted to the GenBank database under accession numbers AF182207 (nucleotides 5392 to 5222) and ABG46345 (Xis).

RESULTS

Identification of the mv4 RDF gene. We previously showed that phage mv4 integrates its genome into the *Lactobacillus delbrueckii* chromosome by site-specific recombination at the 3' end of a tRNA^{ser} gene (22). Prophage mv4 excision seems to involve site-specific recombination between the *attR* and *attL* sites, because after prophage induction by mitomycin C treatment of the lysogenic strain, the cured host strain contained an intact tRNA^{ser} gene (22). Owing to the localization of the RDF gene close to the integrase gene in mobile genetic elements (35), we tried to identify the gene encoding the phage factor promoting excisive recombination by testing various open reading frames (ORFs) located in the close vicinity of the integrase gene: ORF-179, ORF-109, and ORF-57 (Fig. 1). We investigated the possibility that one of these ORFs was able to induce the excision of pMC1 (integrative vector containing the mv4 integrase gene and the *attP* site; Em^r) from the chromosome of *L. plantarum* LP80-3. The three mv4 ORFs were independently cloned into the expression vector pNZ8037 (Cm^r), which was then introduced into LP80-3, and Cm^r transformants were selected. We then screened these transformants for erythromycin sensitivity (Em^s), indicating that the integrated plasmid had been excised and lost, since pMC1 is not replicative in *L. plantarum*. All of the colonies tested (>400) remained Em^r. The mv4 RDF gene was therefore not located in the close vicinity of the integrase gene.

In the early lytic operon, just downstream from the *tec* gene, there is a small ORF encoding a putative basic (pI 9.69) protein containing 56 amino acids (Fig. 1A) (17). Comparison of the sequence of this protein with sequences in protein data-

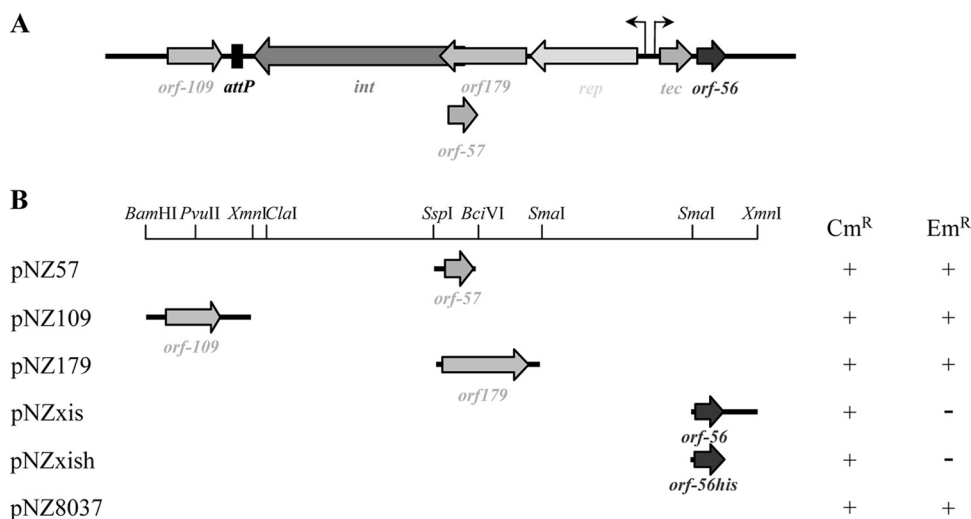


FIG. 1. Identification of phage mv4 RDF gene. (A) Genetic organization of the lysogeny region containing the characterized *rep*, *tec*, and *int* genes, the *attP* site, and ORFs of unknown functions. (B) Series of chloramphenicol-resistant (Cm^r) recombinant plasmids, in which mv4 ORFs are cloned into the expression vector pNZ8037. The cloned gene present in each plasmid is shown, with an arrow indicating its orientation relative to the promoter. Each plasmid was introduced into an *L. plantarum* strain containing an erythromycin-resistant (Em^r) chromosomal integrated vector (pMC1), and chloramphenicol-resistant (Cm^r) transformants were screened for loss of erythromycin resistance (-), i.e., loss of the integrated vector pMC1, as indicated.

bases revealed no significant amino acid identity to any other known RDF. Nevertheless, this ORF displayed weak similarity to the RDF of the SGI1 genomic island from *Salmonella enterica* serovar Typhimurium DT104 (37% identity over a 53-residue segment) (21). Predictions of the secondary structure of ORF-56 by PsiPred (<http://bioinf.cs.ucl.ac.uk/psipred/>) identified a helix-turn-helix (HTH) motif within the N-terminal domain of the protein (data not shown). CLUSTAL X (1.81) analysis of ORF-56, comparing this protein with the proteins from the RDF database (<http://www.pitt.edu/~gfh/download.html>) (35), showed that ORF-56 could be aligned with the SLP1 family, which contains SGI1-Xis (data not shown).

The same test used for ORF-179, ORF-57, and ORF-109 was used to determine whether ORF-56 could induce the excision of pMC1 from the *L. plantarum* LP80-3 chromosome. We found that 100% of the Cm^r transformants (>400) containing the expression plasmid pNZxis had excised pMC1 (Em^s colonies) (Fig. 1B). We carried out the same experiment with the expression vector pNZxish, in which the ORF-56-encoded protein was C-terminally His tagged. Identical results were obtained, demonstrating that the His-tagged protein functioned similarly to the wild-type protein (Fig. 1B). No pMC1 excision event was detected in the absence of mv^4Xis , even if integrant strains were cultured over more than 150 generations in the absence of selective pressure (6).

In vivo, the presence of ORF-56 stimulates the excision of the pMC1 vector that had integrated into the chromosome of a heterologous Gram-positive strain, *L. plantarum*, and this ORF seems to encode a factor that promotes excisive recombination, possibly corresponding to the mv4 RDF, mv^4Xis .

mv^4Xis is absolutely required for excisive recombination. We investigated the site-specific excision reaction mediated by mv^4Xis by developing an *in vitro* excision assay.

The mv^4Xis protein was overproduced in *L. plantarum* by use of a nisin-controlled expression system (45). Following the

induction of *L. plantarum* strains containing pNZxis, we observed the synthesis of a 7-kDa protein, which is absent in induced cells containing the empty expression vector pNZ8037 (data not shown). This protein was produced in relatively small amounts but was completely soluble. The presence of the His-tagged mv^4Xis protein was confirmed by Western blotting with anti-His antibodies (data not shown). In contrast, when the mv^4Xis protein was overproduced in *E. coli* by use of the T7 expression system (pETxis), large amounts of totally insoluble protein were detected for each induction condition, medium, and temperature tested. For these reasons, the *L. plantarum* mv^4Xis cell extract was used in the *in vitro* assay.

The *in vitro* recombination between the *attR* and *attL* sites was monitored by the detection of a radiolabeled linear recombination product resulting from the insertion of a supercoiled *attR*- or *attL*-containing plasmid into a radiolabeled 283-bp or 244-bp DNA fragment containing *attL* or *attR*, respectively. The recombination product, Rec, was detected only in the presence of both mv^4Int and mv^4Xis ; no Rec was detected in reaction mixtures containing only mv^4Int (Fig. 2A and B). The excisive recombination efficiency was Xis dose dependent between 0 and 6 μg of Xis crude extract present in the reaction mixture (Fig. 2A and B). The recombination between *attL* on a plasmid and *attR* on a linear fragment is more efficient (by a factor of two to three) than the reverse situation (Fig. 2A and B). We suggest that the assembly of the higher-order nucleoprotein complex on the *attL* site is favored by this site being supercoiled and/or that the excisive synapsis with the *attR* site is more efficiently constituted. In contrast to the integrative recombination (4), the recombination between the *attL* and *attR* sites on separate linear fragments is effective and is about half as efficient as the recombination between one site on a plasmid and the other on a linear fragment (Fig. 2C).

We also assayed *in vitro* excision activity by monitoring recombination between the *attR* and *attL* sites present on the

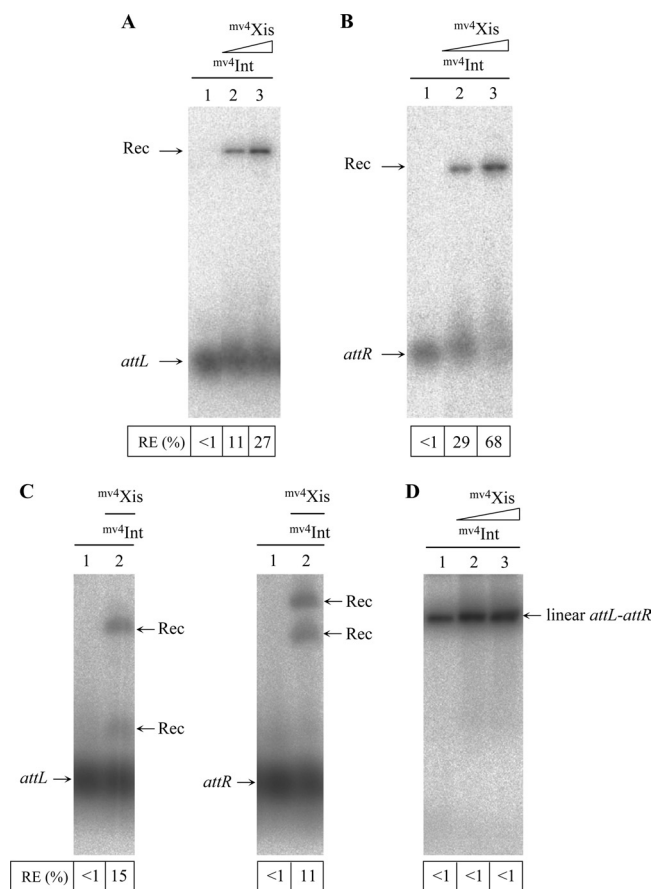


FIG. 2. Role of $mv4Xis$ in *in vitro* $attR$ - $attL$ recombination. Samples were incubated at 42°C for 90 min, analyzed by electrophoresis with a 1% agarose gel, and visualized using a PhosphorImager. The intensities of the bands corresponding to the radioactive recombination product (Rec) and the att substrates ($attL$ and $attR$) were quantified for each sample (as described in reference 4). The recombination efficiency (RE) is indicated under the gels. (A) *In vitro* recombination assays between the $attR$ site-containing plasmid and the linear $attL$ site. Each reaction mixture contained 200 ng of the supercoiled $attR$ plasmid (lanes 1 to 3), the labeled 283-bp linear $attL$ fragment, 3 μ g of $mv4Int$ -enriched cell extract (lane 1), and 3 μ g (lane 2) or 6 μ g (lane 3) of $mv4Xis$ -enriched cell extract. (B) Recombination between the $attL$ site-containing plasmid and the linear $attR$ site. Each reaction mixture contained 200 ng of the supercoiled $attL$ plasmid, the labeled 281-bp linear $attR$ fragment, 3 μ g of $mv4Int$ -enriched cell extract (lane 1), and 3 μ g (lane 2) or 6 μ g (lane 3) of $mv4Xis$ -enriched cell extract. (C) *In vitro* recombination assays between $attR$ and $attL$ sites on linear fragments. Each reaction mixture contained 100 ng of the 2,144-bp linear $attR$ fragment (or the 4,028-bp linear $attL$ fragment), the labeled 283-bp linear $attL$ fragment (or the 281-bp linear $attR$ fragment), 3 μ g of $mv4Int$ -enriched cell extract (lane 1), and 15 μ g of $mv4Xis$ -enriched cell extract (lane 2). (D) *In vitro* recombination assays between the $attR$ and $attL$ sites on a linear fragment. Each reaction mixture contained the labeled 4,134-bp linear pCointRL fragment, 3 μ g of $mv4Int$ -enriched cell extract (lane 1), and 6 μ g (lane 2) or 15 μ g (lane 3) of $mv4Xis$ -enriched cell extract.

same circular DNA molecule, the cointegrate pCointRL, by detecting the two DNA products, the $attP$ -containing plasmid (pRC10) and the $attB$ -containing plasmid (pAMattB). In the presence of $mv4Xis$, the resolution of pCointRL into the two plasmids pRC10 and pAMattB was observed (data not shown). On the contrary, the recombination between the $attL$ and $attR$

sites on the same linear fragment (pCointRL linearized) is inefficient in the presence or absence of $mv4Xis$ (Fig. 2D).

In vivo, in a heterologous Gram-negative host, *E. coli*, in an $mv4Int$ -producing strain, the plasmid pCointRL carrying $attR$ and $attL$ on the same circular DNA molecule was stably maintained in the absence of $mv4Xis$ (Fig. 3A, lane 1). In the presence of $mv4Xis$, the resolution of pCointRL into two plasmids, the $attP$ -containing plasmid (pRC10) and the $attB$ -containing plasmid (pAMattB; not visible because of low copy number), was strongly stimulated and the cointegrate pCointRL was no longer detectable (Fig. 3A, lane 2).

In vivo, in *E. coli*, the recombination between two plasmids carrying $attR$ (pAMattR) and $attL$ (pRCattL) is characterized by the formation of a cointegrate carrying the two resulting $attP$ and $attB$ sites. The cointegrate was detected only in *E. coli* strains containing both pNZ $xish$ and pA3int simultaneously (Fig. 3B), whereas no cointegrate was present in strains expressing only the int gene (data not shown).

In conclusion, $mv4Xis$ is required for the recombination between the $attL$ and $attR$ sites. These experiments were carried out *in vivo* with heterologous Gram-negative or Gram-positive hosts (*E. coli* or *L. plantarum*) or *in vitro* with protein preparations from these hosts, demonstrating that, as integrative recombination (4), excisive recombination either does not require any *L. delbrueckii*-specific accessory factor or requires a universally conserved or substituted factor.

$mv4Xis$ binds specifically to the $attR$ and $attP$ sites. Generally, RDFs control recombination by binding to the attachment sites. $mv4Xis$ probably affects $mv4$ recombination in a similar way, because it contains a putative DNA-binding domain (the HTH motif). To demonstrate this property, we investigated the ability of $mv4Xis$ to specifically retard the migration of substrates for excisive recombination, $attL$ and $attR$, and substrates for integrative recombination, $attB$ and $attP$, on gel electrophoresis.

Following the incubation of $mv4Xis$ -enriched cell extract with $attR$ or $attL$ DNA fragments, two retarded complexes were detected with $attR$ and none with $attL$ (Fig. 4B). When this experiment was carried out with $attB$ and $attP$, we observed two retarded complexes with $attP$ and none with $attB$. No complex was formed with any of these sites following incubation with control cell extract (pNZ8037) (Fig. 4B). These results indicated that $mv4Xis$ binds to the P arm and not to the P' arm of the attachment sites.

$mv4Xis$ binds to two distinct regions in the P arm of the $attR$ and $attP$ sites. The presence of two retarded complexes suggests that two Xis binding sites may be available in the $attP$ and $attR$ sites. Deletion analysis of the $attP$ substrate by electrophoretic mobility shift assays in the presence of $mv4Xis$ identified two independent regions likely to contain such binding sites (Fig. 4, compare $attPD4$, $attPBB$, and $attPBX$). Analysis of the sequences of these two regions, extending from positions -99 to -80 and from positions -47 to -21, respectively, led to the identification of a 5'-GAAACA sequence present in both regions (Fig. 5A). These two 6-bp sequences, designated X1 and X2, are separated by 54 bp and are in direct orientation. They are located on the same side of the DNA, separated by six turns of the DNA helix, between the P1 and P2 putative Int -binding sites. The X1 binding site is 13 bp away from the P1 site, and the X2 site is adjacent to the P2 site (Fig. 5A).

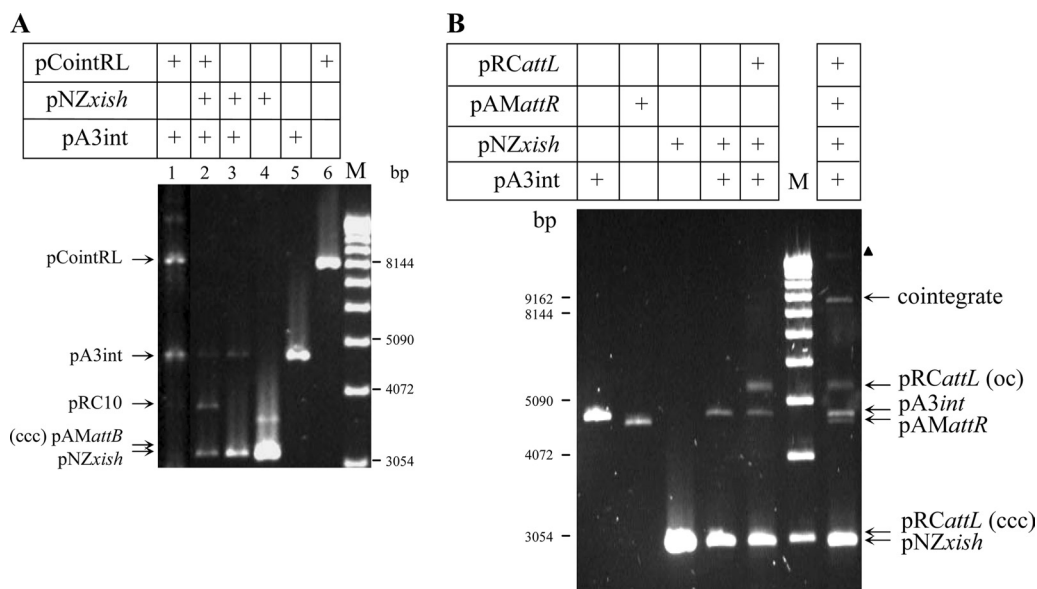


FIG. 3. Role of *mv4*Xis in *in vivo* *attR-attL* recombination. (A) In *E. coli*, the recombination between the *attR* and *attL* sites placed on pCointRL is visualized by the resolution of pCointRL and the formation of the resulting plasmids pRC10 and pAMattB in the presence of Int (pA3int) and Xis (pNZxish). Plasmids isolated from the *E. coli* strains were digested with EcoRI, which linearizes pA3int, pNZxish, pRC10, and pCointRL but not pAMattB. The resulting fragments were separated by electrophoresis on a 0.8% agarose gel, which was stained with ethidium bromide. The arrows indicate the locations of the different plasmids. pAMattB was not detectable because of the low copy number. Lane M, 1-kb DNA ladder (Invitrogen). (B) In *E. coli*, the intermolecular site-specific recombination between the *attR* and *attL* sites placed on pAMattR and pRCattL, respectively, was visualized by the formation of a cointegrate plasmid carrying the resulting *attB* and *attP* sites. Plasmids isolated from the *E. coli* strains were digested with BstBI, which linearizes pAMattR, pA3int, pNZxish, and the cointegrate but not pRCattL. The resulting fragments were analyzed as described for panel A. The black triangle indicates the possible multimeric form of pRCattL or an additional recombination product (cointegrate + pRCattL). Lane M, 1-kb DNA ladder (Invitrogen). oc, open circular; ccc, covalently closed circular.

We investigated whether this motif constituted the binding site for *mv4*Xis by introducing multiple mutations into X1 or X2 to yield *attP* sites with potentially defective Xis-binding sites (X1mut and X2mut, AACCCG [where the mutated residues are underlined]). The effects of these changes were evaluated by electrophoretic mobility shift assays with *mv4*Xis (Fig. 4). When the *attP* substrate containing one of the two mutated binding sites (*attPX1mut*) was incubated with an *mv4*Xis-enriched cell extract, only one complex was detected. This is consistent with there being only one site available, X2 (Fig. 4). When the mutated X2 binding site was tested with the truncated *attP* site *attPX2mut* (Fig. 4A), no retarded complex was detected (Fig. 4B).

The same experiments were performed with the equivalent *attR* derivatives, and the results confirmed those obtained with the *attP* site and its derivatives (data not shown).

***mv4*Xis bends the P-arm DNA.** Evidence that *mv4*Xis induces a bend in the DNA at the P arm was obtained by analyzing the relative mobilities of *mv4*Xis-DNA complexes with a set of *attR* substrates circularly permuted for the X2 site (Fig. 6A). *mv4*Xis formed detectable retarded complexes with five of the substrates, and the relative mobilities of the protein-DNA complexes are consistent with a Xis-induced DNA bend (Fig. 6B). *mv4*Xis did not form complexes with two of the DNA substrates, generated with restriction enzymes cutting close to the X2 site (Fig. 6A). The approximate bending angle was estimated at 90° (54).

***mv4*Xis does not inhibit Int-mediated integrative recombination.** We investigated the role of *mv4*Xis in integrative site-

specific recombination between the *attP* site and the *attB* site but also between the *attP* site and the *attR* or the *attL* site, which can be secondary integration sites. We previously set up an *in vitro* integration reaction in which *mv4*Int efficiently catalyzes recombination between the *attP* and *attB* attachment sites (4).

Comparisons of the *in vitro* integrative recombination efficiencies of reactions between an *attP*-containing plasmid and the radiolabeled linear fragments containing the *attB*, *attL*, or *attR* site showed that the *attL* site was essentially equivalent to *attB* in recombination with *attP* (Fig. 7A). Conversely, the recombination efficiency of the reaction between the *attP* and *attR* sites was about a quarter as efficient as that between the *attP* and *attL* sites (Fig. 7A). In experiments carried out with the *attL* or *attR* site on a plasmid and the *attP* site on a linear radiolabeled fragment, the recombination efficiencies were similar to those previously obtained with the reverse locations (data not shown).

Whatever the substrate used for recombination with the *attP* site, the integrative recombination efficiency is not affected by the presence of *mv4*Xis (Fig. 7A).

Similarly, no marked effect on the time course of the integrative recombination reaction was observed in the presence of *mv4*Int and two different concentrations of *mv4*Xis compared to the time course of the reaction in the presence of *mv4*Int alone (Fig. 7B). In addition, the intermolecular recombination efficiency between the *attP*-containing plasmid and the radiolabeled linear fragment containing *attB* was analyzed in the presence of various ratios of *mv4*Xis/*mv4*Int (up to 50). Compared to

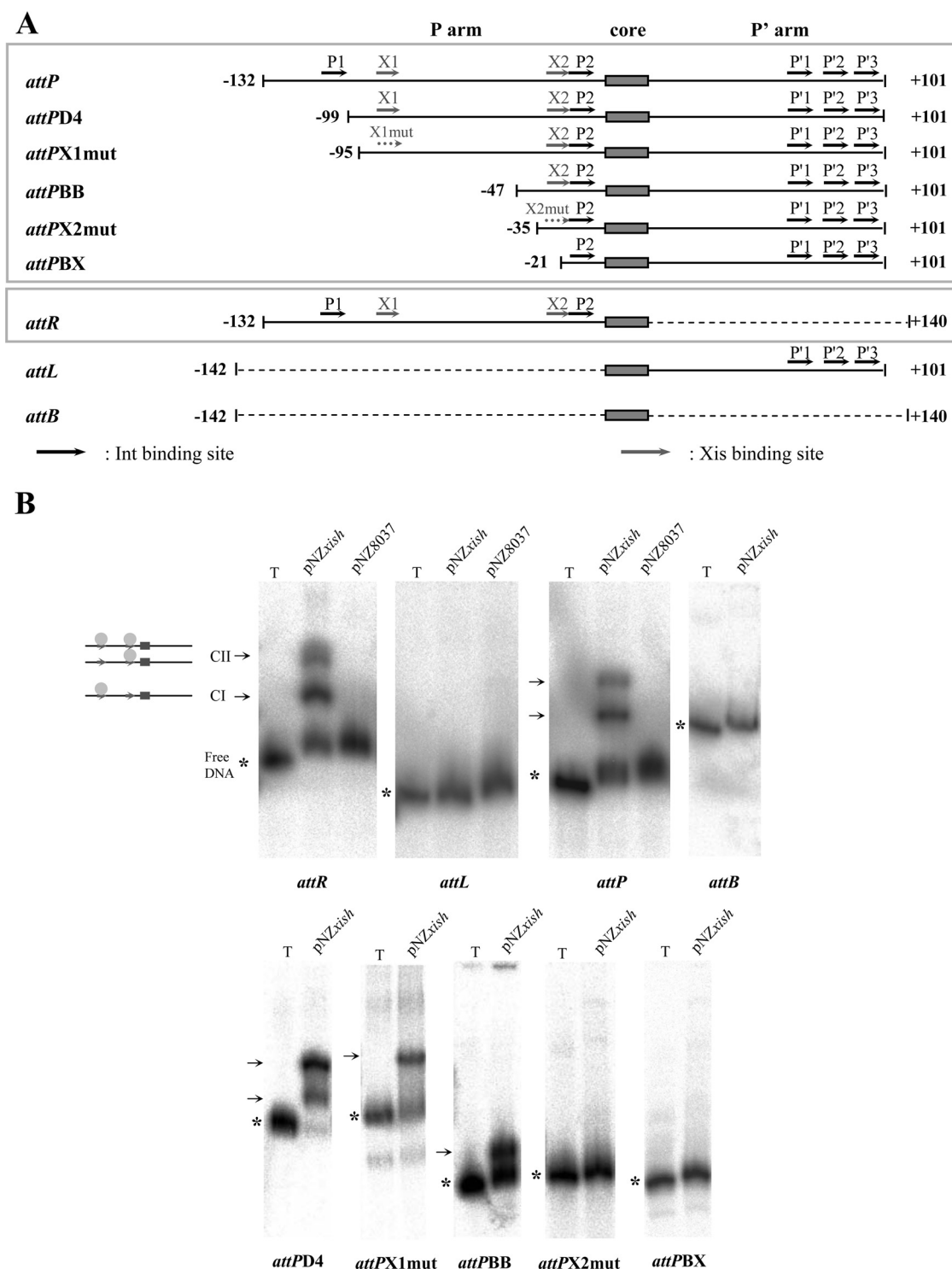
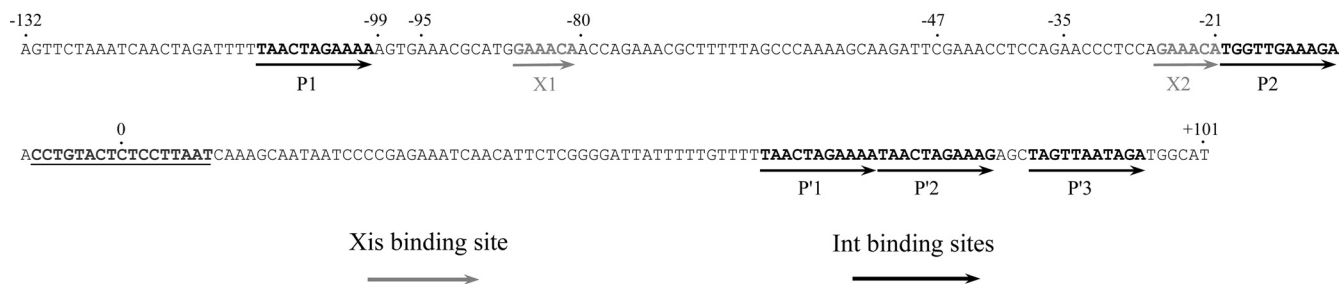


FIG. 4. ^{mv4}Xis binds to *attR* and *attP* DNA. (A) Schematic representation of *attR*, *attL*, *attB*, and deletion derivatives of the *attP* sites used as radiolabeled fragments for the experiment shown in panel B. The solid horizontal lines designate the *attP*-derived DNA and the dashed lines the *attB*-derived DNA. The numbers at both ends indicate the first and last bases of the segment according to the *attP* region (4). The 17-bp core sequence is indicated by the shaded box. (B) Gel electrophoresis of protein-DNA complexes. Linear radiolabeled fragments (indicated under the gels and diagrammed in panel A) were incubated with 10 μ g of an ^{mv4}Xis-enriched cell extract (pNZxish) or with 10 μ g of a control cell extract (pNZ8037). The protein-DNA complexes were analyzed by electrophoresis with a 5% polyacrylamide gel. In lane T, only the radiolabeled fragment was included in the reaction mixture, without cell extract. The linear radiolabeled fragments are indicated with an asterisk (*). The complexes are indicated with arrows, and the drawings to the left of the complexes represent the different configurations of ^{mv4}Xis binding.

A



B

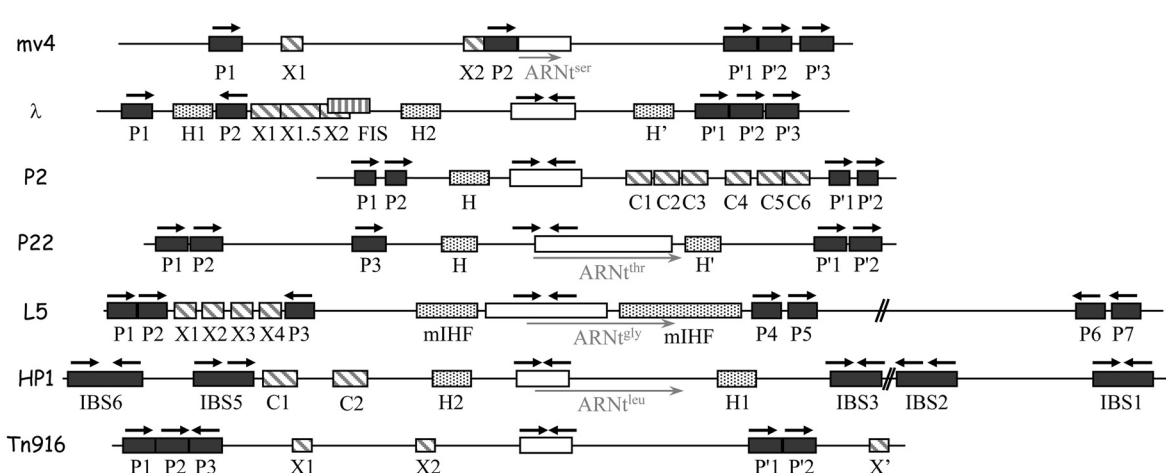


FIG. 5. Organization of the *attP* binding sites. (A) Structure and nucleotide sequence of the mv4 *attP* region. The numbers above the sequence correspond to the boundaries of the corresponding *attP* sites presented in Fig. 4A (except 0, which corresponds to the central base of the core region). The core sequence is underlined. The black arrows indicate the 11-bp consensus sequence 5'-TRRYTRRWARR-3', which is represented five times in *attP*, and the gray arrows indicate the 6-bp sequence of the Xis-binding site. (B) Comparison of the *attP* site organizations of λ , P2, P22, L5, HP1, and mv4 phages and the Tn916 transposon (according to references 18, 25, 36, 39, 44, 48, and 57). Integrase binding sites are represented by black squares, IHF binding sites by gray plotted squares, Xis binding sites by gray diagonally striped squares, and the FIS binding site by a gray vertically striped square. The gray arrows represent the 3' end of the tRNA gene. The core site is represented by a white square, and the black arrows indicate the orientations of the Int arm-type and core-type binding sites.

the recombination efficiency in the presence of various ratios of control cell extract^{mv4}Int (up to 50), no significant inhibition was observed (Fig. 7C).

In the *in vitro* test used here, in the presence of both ^{mv4}Int and ^{mv4}Xis, the recombination reaction remains unidirectional, i.e., integrative, since the resulting *attL* and *attR* sites neoformed by the *attP-attB* recombination reaction are present on a linear DNA fragment and the intramolecular *attL-attR* recombination is not detectable *in vitro* in this case (Fig. 2D). Therefore, the results observed are only due to the effect of ^{mv4}Xis on the sole integrative recombination.

DISCUSSION

We have demonstrated that mv4 ORF-56 encodes the phage RDF, a 56-amino-acid protein required for efficient site-specific excisive recombination. The amino acid sequence of mv4 RDF does not resemble that of the phage lambda excisionase but is distantly related to the RDF of the L5-SAM-SPL1 family, especially to the SGI1 and SLP1 RDF (35). Otherwise, mv4Xis shares several common characteristics with the other RDF, such as a small size and a basic pI (35). mv4Xis appears to have a function similar to that of the lambda Xis, in that it

is required for excisive recombination, but mv4Xis differs from the lambda Xis in that it seems not to inhibit integrative recombination. This is the main difference between this system and other previously described excisive recombination systems involving the RDF (2, 12, 24, 36).

The mv4 RDF is encoded by the second gene of the early lytic operon, downstream from the P_{tec} promoter, whereas the *int* gene is the third gene of the lysogenic operon transcribed from P_{rep} (17). To our knowledge, this is the first time that such a position has been reported for an RDF gene in a nondefective temperate bacteriophage harboring tyrosine recombinase. Usually, for the majority of the phages, particularly for the lambdoid phages, the *xis* gene is in the immediate vicinity of the *int* gene and is often located in the same operon (35). In nonlambdoid bacteriophages of the P2 family and in phage HP1, the gene encoding the RDF, *cox*, is the first gene of the early lytic operon (24, 57), but in this case, Cox has an additional function, regulating the activity of the promoters involved in the genetic switch between lysis and lysogeny (26, 50). The two functions of the Cox protein are carried out by two distinct proteins, Tec and Xis, in phage mv4 (17).

The presence of the *xis* gene in the early lytic operon may

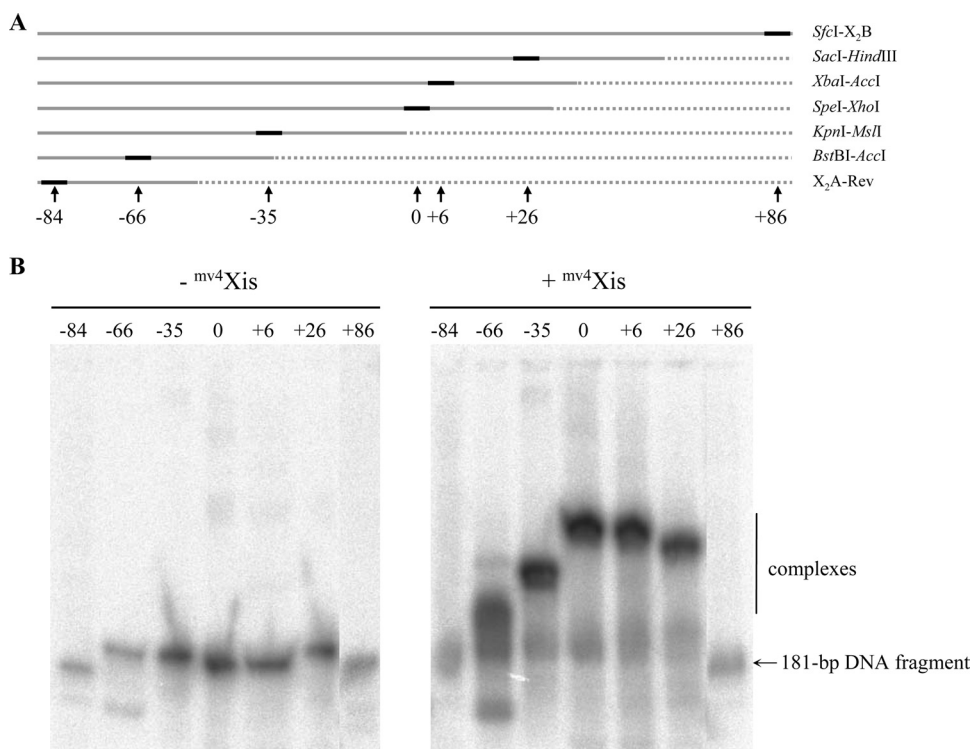


FIG. 6. $mv4Xis$ bends the P-arm DNA. (A) Seven circularly permuted DNA fragments containing the X2 binding site of $mv4Xis$ were generated by cleavage of pRCattRDR2 with different enzymes, generating 181-bp fragments. The short black line represents the X2 binding site, and the long gray line represents the circularly permuted DNA fragments. The numbers under the arrows indicate the relative locations of the X2 binding site with respect to the center of the fragment (0). (B) These labeled fragments were incubated with the $mv4Xis$ -enriched cell extract (+ $mv4Xis$) or with the control cell extract (- $mv4Xis$), and the DNA-protein complex mobility was analyzed by gel electrophoresis.

reflect the regulation of recombination via the regulation of *xis* and *int* expression. Indeed, the recombination directionality factor, antagonizing the integration process, should be expressed only during induction of the lytic cycle and would therefore need to be tightly regulated. The *mv4 int* and *xis* gene localization implies that the expression of the two genes is not coregulated during the *mv4* phage cycle. In the lysogenic strain, the *int* gene is transcribed at a low level from the P_{rep} promoter, whereas the early lytic operon with the *xis* gene is totally repressed. During prophage induction, the transcription of the lysogenic operon with the *int* gene is shut off and the lytic operon is transcribed from the P_{tec} promoter at a high level, leading to extensive synthesis of Xis (17). In P2-like phages and in phage TP901-1, where the overall genetic organization of the genetic switch resembles that described for *mv4* (17, 37, 38, 50, 57), the regulation of the site-specific recombination genes is achieved in a similar way. In the lambdoid phages, the *int* and *xis* genes are present in the same operon. When both the integrase and the RDF are required, the transcription of the two genes is initiated from the major early P_L promoter (23). During lysogenization or in a lysogenic strain, *int* transcription is initiated at a promoter, P_I , located in the *xis* gene (28).

We investigated the role of $mv4Xis$ in excisive recombination. In the presence of $mv4Int$, we observed an absolute requirement of $mv4Xis$ for recombination between *attR* and *attL* to occur both *in vivo* and *in vitro*. We also noted a significant influence of the supercoiling state of the recombination substrates on the excisive reaction. Although the linear *attL* and

attR substrates proved to be totally competent for recombination, the reaction efficiency was strongly enhanced with at least one supercoiled substrate, regardless of which one was provided in circular form. This contrasts with the integrative reaction, in which the superhelicity of the *attP* site is required and linear substrates are not efficient (4).

As was shown for the integrative reaction with $mv4Int$ (4), the excisive reaction seems independent from host accessory factors, like IHF or FIS for the lambda recombination system, since the experiments were performed *in vivo* with various heterologous bacterial hosts (*E. coli* and *L. plantarum*) or *in vitro* with cell extracts of these hosts. However, we cannot exclude the requirement of at least one accessory factor universally present in these strains. The independence of host accessory factors in the site-specific recombination reaction is unusual.

We have shown that $mv4Xis$ binds to and bends *attR* and *attP* DNA. The two Xis-binding sites are located on the P arm of *attR* or *attP* between the putative $mv4Int$ -binding sites P1 and P2, without overlapping these sites (Fig. 5A). In the P arms of the various phages analyzed, the number of the P-arm binding sites of the integrase is relatively constant (2 or 3), but between the Int arm-binding sites and the core sequence, multiple binding sites for host and phage DNA bending proteins are present (Fig. 5B). Compared to other phage systems (Fig. 5B), the recombination protein binding site organization on the P arm is unique for *mv4* due to the absence of host or phage DNA bending protein binding sites between the Int arm-binding sites and the core sequence, suggesting a specific, less complex P-

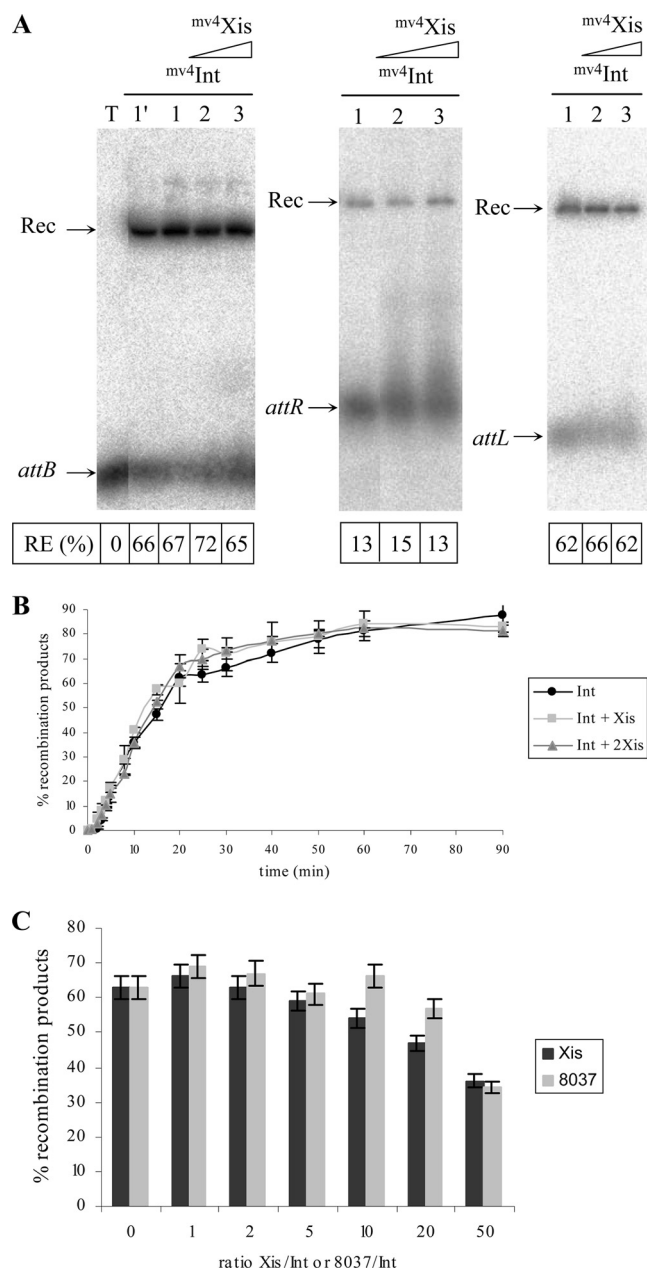


FIG. 7. Role of *mv4*Xis in *in vitro* integrative recombination. The samples and the recombination efficiencies (RE) were analyzed as described in the legend for Fig. 2. (A) *In vitro* recombination assays between linear radiolabeled fragments containing the *attB*, *attL*, or *attR* site and the *attP*-containing plasmid. Each reaction mixture contains the linear *attB*, *attL*, or *attR* fragment, 200 ng of the supercoiled *attP* plasmid, 3 μ g of *mv4*Int-enriched cell extract (lanes 1' and 1 to 3), and 3 μ g (lane 2) or 6 μ g (lane 3) of *mv4*Xis-enriched cell extract or 6 μ g of control cell extract (pNZ8037) (lane 1'). The recombination efficiencies are indicated under the gels. (B) Kinetics of *attP* \times *attB* recombination in the presence of Int or Int and Xis. The reactions were realized as described for panel A. At the indicated times, aliquots were removed, and the reactions were stopped by adding 0.1% SDS. The recombination efficiencies are plotted on the graph. (C) *In vitro* *attP* \times *attB* recombination assays in the presence of Int (1 μ g in each reaction mixture) and various amounts of the control cell extract (8037) or the *mv4*Xis-enriched cell extract (Xis). The ratios of Xis/Int or 8037/Int are shown below the graph. The reactions were realized as described for panel A, and the recombination efficiencies are plotted on the graph. Error bars indicate standard deviations.

arm folding mechanism. The binding of *mv4*Xis to X1 and X2 might bend the DNA P arm by about 180° to bring the P1-arm site close to the core sequence, thereby facilitating the bridging of these two sites (P1-arm site–core sequence) by a bivalent DNA-binding integrase monomer. Based on these observations, *mv4*Xis may play at least an architectural role, influencing excisive intasome assembly on *attR*. This situation evokes that of Xis-L5. In L5 directionality control, Xis-L5 has only an architectural role, with the direction of recombination controlled exclusively by means of the binding and bending of DNA (36). Xis-L5 promotes excision by facilitating the formation of the *attR* intasome, in which Xis-L5 indirectly enhances Int-L5 occupancy of the P1/P2 arm-type sites by assisting in the formation of intramolecular bridges between the P1/P2 sites and the core (36). In phage λ , Xis controls the directionality of recombination by stimulating the excisive reaction while simultaneously inhibiting the integrative recombination. These dual and opposing effects are realized in two ways. First, the cooperative binding of three Xis monomers to the P arm forms a micronucleoprotein filament (1), which substantially bends *attR* DNA and alters its trajectory within the excisive intasome, thereby stabilizing the synaptic complex in which *attL* and *attR* are bridged by bivalent DNA-binding Int proteins (30). Second, Xis cooperatively recruits an integrase monomer to the P2-arm site through protein-protein interactions at its C terminus (13, 56) and therefore inhibits the formation of a proficient integrative intasome on *attP*. Due to the close proximity of the P2-arm site and the X2 *mv4*Xis binding site in the *mv4* P arm, we cannot exclude interactions between *mv4*Xis and *mv4*Int in order to facilitate the formation of the excisive synapsis.

We have shown that *mv4*Xis does not inhibit integrative recombination between the *attP* site and the *attB*, *attL*, or *attR* site under our experimental conditions (Fig. 7). The lack of inhibition by *mv4*Xis of the integration reaction would be an unprecedented behavior among characterized recombination directionality factors. Indeed, in all of the site-specific recombination systems in which RDFs have been described, even if the RDF genes are expressed from the lytic operon, as with P2-like phages, recombination between *attP* and *attB* has always been reported to be inhibited, in various ways, by the RDF (7, 24, 27, 36, 41, 57). For P2 (57) or λ (2, 41) phage, the RDF concentration completely inhibiting the integrative recombination is the same as that necessary for maximal stimulation of excision. For the two phages, the integrative recombination inhibitory effect is observed with a ratio of Xis/Int of about 0.5 to 1 in the *in vitro* reaction. The *in vitro* *mv4* experiments used to test the inhibition of the integrative recombination were realized under the same conditions. Moreover, we have tested *mv4*Xis/*mv4*Int ratios up to 50 without inhibition of the integrative recombination.

The formation of the integrative intasome on the *mv4* *attP* site seems to be unaffected by *mv4*Xis, as are the *attP* intasome interactions with the *attB*, *attR*, and *attL* sites. The higher-order nucleoprotein complex assembly on the P arm in the *attP* intasome probably involves a bridge to be formed between the P1-arm site and the core region by an *mv4*Int monomer, and this structure may be stabilized by protein-protein interactions with the three *mv4*Int monomers assembled on the P' arm to get a functional intasome. However, this structure may be unstable when formed on the *attR* site, because of the absence

of the P' arm. The binding of $mv4$ Xis to the P arm and the subsequent DNA bending might then be required for the stabilization of this *attR* intasome and its assembly into the excisive *attL-attR* synapsis. The P'-arm nucleoprotein complex on *attL*, the *attL* intasome, seems to be stable independently of the presence of the P arm or $mv4$ Xis. Thus, $mv4$ Xis may play a purely architectural role, allowing the higher-order nucleoprotein complex to assemble on *attR* and facilitating the formation of the excisive synapsis. This function seems to have no inhibitory effect on integrative synapsis assembly involving the *attP* and *attB* (or *attL* or *attR*) sites.

During phage infection or prophage induction, inhibition of the integrative reaction is not mandatory to avoid phage (re)integration events and to get an efficient lytic cycle. Due to the specific genetic organization of the recombination system in *mv4*, in both cases, there is a P_{tec} -driven burst of transcription of the early lytic operon, which leads to overproduction of $mv4$ Xis and Tec, whereas at the same time the lysogenic operon expression is very low, due to nonactivation by Rep or repression by Tec, conditions allowing the presence of a basal level of integrase sufficient to catalyze excision.

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